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EXPERIMENTAL STUDY OF THE DISTRIBUTION AND HABITAT OF THE TETANUS BACILLUS *

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In this work an attempt has been made to determine the importance of some of the herbivorous animals as carriers or distributors of tetanus spores. With this object in view, the feces of many animals have been examined and the following points have been emphasized: How many of the animals harbor the organism in the intestines? To what extent are tetanus spores present in the feces of such animals? Do such animals become permanent carriers, or do they eventually rid themselves of the organism? Can the tetanus bacillus multiply in the animal intestine?

HISTORICAL REVIEW

The tetanus bacillus appears to be of almost universal distribution in nature. Lortet[1] found it in the mud dredged from the bottom of Lake Geneva as well as in the waters of the Dead Sea. Ledantec[2] reports of having found it in the poisoned arrows of the natives of the New Hebrides, who obtained it by smearing their arrow heads with the material found in the burrows of large crabs. Peyraud[3] has found it in 50 percent of the samples of hay dust examined by him, and Ringeling[4] reports its presence in the bilge water of ships. The investigations of many other workers show it to be frequently present on wearing apparel,[5] upon the skin of man and animals, in gun wads, and in different soils. Nicolaier[6] found it in twelve out of eighteen samples of soil collected about Göttingen, and of one hundred and ninety-two specimens from Leipzig, Berlin, and Wiesbaden, eighty-one showed it when inoculated into mice. Bossano[7] in Marseilles tested the soils of thirty-eight cities in different parts of the world, and samples from only twelve of the cities were free from it.

Generally, when the bacillus has been found in soils, it has been garden soil, especially soil fertilized with animal feces, or dust and dirt from streets. In his own work, Nicolaier observed that the samples containing the bacillus came from gardens, court-yards, streets, or riding halls, but that unfertilized soil did not contain it. Bissérie[8] likewise found earth contaminated with animal feces to be highly infected with tetanus spores, while forest soils were not.

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1. Centralbl. f. Bacteriol., 1891, 9, p. 709; Ibid., 10, p. 567.
2. Ann. de l'Inst. Pasteur, 1890, 11, p. 716.
3. Centralbl. f. Bakteriol., 1891, 9, p. 17.
4. Quoted in Kolle and Wassermann: Handbuch der pathogenen Mikroorganismen.
5. Centralbl. f. Bakteriol., 1908, 41, p. 390.
6. Inaug. Diss., Göttingen, 1885; abs., Baumgarten's Jahresbericht, 1886, 2, p. 270.
7. Quoted in Kolle and Wassermann: Handbuch der pathogenen Mikroorganismen.
8. **Ibid.**

Sormani,[9] in 1899, found tetanus bacilli in the freshly deposited feces of many animals, and Chicoli Nicola[10] found them to be especially abundant in horse feces. Sormani[11] and others further showed that tetanus is not produced in animals by feeding the organism, but that the feces of such animals will subsequently contain it. Pizzini[12] found the bacillus in 5 percent of the human feces examined. His figures are striking, for they show that 30 percent of the men working about horses and stables have tetanus bacilli in their feces, while only 2.2 percent of men engaged in other occupations have them.

The frequent occurrence of the bacilli in the animal intestine is highly suggestive. It is readily conceivable, when we consider the wide distribution of the spores in the soil, hay-dust, grain, etc., that their normal mode of entry into the digestive tract is by ingestion with food, or through breathing and swallowing infected air. That this may occur in the case of the human intestine has been shown by Pizzini,[12] and more recently by Freund[13] in the report of a case of puerperal tetanus, in which the patient was undoubtedly infected through her own feces. Whether the spores that are swallowed remain inactive, provided they do not obtain a lodgement in a wound or lesion of the digestive tract, and are quickly eliminated in the feces, or whether they effect a permanent, or even a temporary, foothold in the intestine is a question which has led to much controversy. Thus, Sormani,[14] after finding the bacillus in the feces of many animals, advanced his fecal theory to explain the universal distribution of the organism. He believes that passage through the digestive tract is a necessary phase in the life activities of the bacillus, that in the intestine it multiplies and renews its virulence, and that its spores are then passed out in the feces and so spread over the surface of the ground. He believes that it cannot multiply in the soil because of unfavorable meteorological conditions, such as light and the lack of moisture, that it gradually loses its virulence there, and that only the extreme resistive powers of its spores enable it to survive. On the other hand, with the absence of oxygen, with the favorable temperature and rich food supply in the animal intestine, conditions should be most favorable for its growth.

The other side of this controversy has been taken by Vincent[15] with his "telluric theory." While admitting the presence of the tetanus bacillus in the intestines of many of the herbivora, he doubts that it multiplies there since the feces of all of the animals he examined did not show it. He has fed rabbits small amounts (1 c.c.) of spore-containing cultures and killed the rabbits at the end of twelve hours. He claimed that microscopic examinations and animal inoculations from cultures made along different parts of the alimentary canal showed no increase in the number of tetanus bacilli. In another series of experiments, he reports feeding tetanus spores to rabbits and guinea-pigs in which the pylorus was ligated, and he finds that "the number of spores in the stomach is greatly diminished by the end of two hours." He finally shows that tetanus bacilli will not grow readily in vitro when planted in pancreatic extract, or in the extract from the intestinal wall; furthermore, "bile shows an antiseptic action" on them. Accordingly, he believes that they must grow in decomposing organic matter, such as the rotting straw and manure in the stable, where they are protected from the light, and where moisture and the heat of decomposition, together with the presence of many aerobic bacteria which absorb oxygen, offer favorable conditions.

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[9] Centralbl. f. Bakteriol., 1890, 7, p. 250.
[10] Ibid., 1891, 9, p. 18.
[11] Ibid., 1891, 10, p. 421.
[12] Baumgarten's Jahresbericht, 1894, 14, p. 236.
[13] Ztschr. f. Geburtsh. u. Gynäk., 1912, 72, p. 97.
[14] Centralbl. f. Bakteriol., 1892, 12, p. 609
[15] Comp. rend. Soc. de biol., 1908, 65, p. 12.

His evidence is not entirely conclusive. That he could not detect any increase in numbers twelve hours after the feeding of spores to a rabbit does not seem strange if we consider, first, the difficulty of making even an approximate numerical comparison between the spores fed and those recovered from the intestine, and, second, that even under the most favorable conditions of artificial cultivation we can never detect growth of tetanus bacilli as early as twelve hours after planting, and only exceptionally can we do so by the end of twenty-four hours. That the number of spores in the stomach with the pylorus ligated should be greatly reduced two hours after feeding does not seem at all peculiar in view of the abnormal conditions and the long exposure to the acidity of the gastric juice. He advances no direct experimental evidence that the tetanus bacilli multiply in decomposing organic matter, and in opposition to his theory that they do so is the evidence of Bombicci[16] and of Von Esmark[17] that these bacilli quickly disappear from putrefying mixtures, and that their spores can be demonstrated in the surrounding material for only a short time thereafter.

The conflicting evidence thus presented by Vincent and Sormani as to the manner of growth and multiplication has made it desirable to undertake the work herein reported in the hope that different technic and methods of cultivation might throw further light upon this subject.

METHODS OF CULTIVATION AND EXAMINATIONS

As a preliminary to the actual experimental work, many methods were tested for the purpose of finding one for cultivating tetanus bacilli which would be both reliable and simple. Smith's[18] tissue method for cultivating anaerobes, as modified by Tarozzi,[19] was eventually selected as one well suited to the requirements of this work. The medium was prepared as follows:

Test tubes were filled with the ordinary Liebig's beef extract nutrient broth and sterilized in the usual way. The tissues of young and healthy white rats, guinea-pigs, rabbits, and cats were used. The animals were chloroformed, thoroughly washed with a 1:1,000 solution of mercuric chlorid, the skin stripped from the abdomen, and the latter opened with sterile instruments. The liver and kidneys were removed from the peritoneal cavity, with all aseptic precautions, to large covered Petri dishes prepared to receive them, and care was taken to avoid the gall-bladder while removing the liver. Small pieces (approximately 1 gm. of tissue for each 10 c.c. of broth) were then cut off and dropped into the broth tubes. These were then incubated for two days, when they were ready for use. By this method, with reasonable care, there is little danger of contamination, and the period of preliminary incubation is of advantage in that tubes which may have been contaminated will show growth and may be rejected.

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[16] Baumgarten's Jahresbericht, 1891, 7, p. 220.
[17] Ztschr. f. Hyg. u. Infectionskrankh., 1889, 7, p. 1.
[18] Centralbl. f. Bakteriol., 1890, 7, p. 502; Jour. Med. Research, 1906, 14, p. 193.
[19] Centralbl. f. Bakteriol., 1905, 38, p. 619

Routine Examinations.—The feces of sixty-one horses, twenty-one cows, and one guinea-pig were examined for tetanus spores.

The feces were collected immediately after being passed and brought to the laboratory. Material from the center of the fecal masses in the cases of the horses and cows was weighed out in sterile Petri dishes. Examinations were made of both unheated and heated feces. The unheated feces, after being weighed out in 10 gm. and 1 gm. lots, were planted directly in Smith's[18] tissue broth in the proportion of an approximate 10 percent suspension and then incubated at 37 C. The feces which were to be examined in a heated condition were first planted in tubes of plain broth, in a 20 percent suspension, and the tubes then placed in the water bath at 80 C. for ten minutes. After being cooled to 40 C. an equal volume of tissue broth was added, and the cultures then incubated at 37 C. This procedure was adopted to avoid heating the tissue broth, since heat destroys the efficacy of the tissue to support the growth of anaerobic bacteria. At the expiration of from six to ten days, smears were made from both unheated and heated cultures, stained by Gram's method, and examined for tetanus-like, terminal, spore-bearing bacilli. From the cultures showing such forms, young white rats were inoculated with 1 c.c., and from those rats dying with typical symptoms of tetanus the bacillus was recovered by dropping bits of tissue or of pus from the site of inoculation into tissue broth tubes, or by scraping the wound with a platinum loop and inoculating tissue broth tubes. Cultures thus made were grown for one week and then tested by inoculating them into white mice.

Of the sixty-one horses examined, eleven, or 18 percent, showed tetanus bacilli in their feces. No tetanus bacilli were found in the feces of the cows. Park[20] states that 15 percent of horses and calves in the vicinity of New York City harbor tetanus bacilli in their intestines. Sanchez-Toledo and Veillon[21] obtained tetanus bacilli from the feces of four horses out of six examined, and of one cow, the only one tested. It would seem that our figures compare favorably with those given by Park[20] for horses. Sanchez-Toledo and Veillon[21] have examined but few animals, and hence it is unfair to compare our results with theirs.

Our examinations of cow feces were made during the winter months at a time when the animals were confined in a barn (all the animals belonged to one dairy), and when their diet was largely of ensilage, a highly fermented food and therefore one not likely to contain tetanus spores. Some of our results seem to indicate that the tetanus bacillus, when planted in the intestines of certain individual animals, will remain there but a short time, being eventually eliminated. This may explain our failure to find the organism in cow feces.

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[20] Pathogenic Bacteria and Protozoa, 1910, p. 232.
[21] Centralbl. f. Bakteriol., 1891, 9, p. 18.

The feces of one guinea-pig were examined for tetanus bacilli, and rats and mice that were inoculated died of tetanus in forty-eight hours. Sanfelice[22] has reported the recovery of tetanus spores in the feces of seven normal guinea-pigs out of twenty-three examined by him.

Quantitative Examinations.—To determine to what extent the tetanus bacillus is present in the feces of animals which have been shown to harbor it within their alimentary canals, quantitative bacteriological examinations of the feces were made. For this purpose "Nora" and "Lady," two horses known to harbor the organism, were used. These quantitative determinations were made as follows:

One gram of horse feces from the interior of a fecal ball was planted in 10 c.c. of plain broth. This suspension was placed in an automatic shaker for one hour to obtain a uniform emulsion. Dilutions of 1:10, 1:100, 1:1,000, 1:10,000, and 1:100,000 were then made successively by transferring 1 c.c. of this fecal emulsion into 9 c.c. of tissue broth, and so on. The experiment was done in duplicate; one of the tubes containing the initial suspension of feces was heated to 80 C. for ten minutes before the dilutions in the tissue broth were made. After incubation of the culture tubes for six days, inoculations were made into young white rats. The results are recorded in Table 1.

TABLE 1

SHOWING RESULTS OF QUANTITATIVE EXAMINATION OF FECES OF TWO HORSES CARRYING TETANUS BACILLI

	February 10—"Lady"		February 25—"Nora"	
Amount, in Gm., of Feces Tested	Cultures Tested Without Preliminary Heating	Cultures Heated 80 C. for 10 Minutes Before Inoculation	Cultures Tested Without Preliminary Heating	Cultures Heated 80 C. for 10 Minutes Before Inoculation
1	+	+	+	+
0.1	+	0	+	+
0.01	0	0	0	0

From these figures it seems either that tetanus bacilli are not present in large numbers in the intestine of the horse or our present-day methods of making quantitative bacteriological determinations, at least in the case of anaerobes, are not sufficiently accurate and reliable to detect an isolated spore or a small group of organisms.

FEEDING EXPERIMENTS

Two sets of experiments have been carried out for the purpose of determining whether or not an animal the intestines of which have once been infected with tetanus spores becomes a permanent carrier.

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[22] Ztschr. f. Hyg. u. Infectionskrankh., 1893, 14, p. 234.

Guinea-pigs have been given single feedings of relatively large amounts of cultures containing tetanus spores, and their feces examined subsequently at frequent intervals.

These experiments were divided into two periods: A fore-period, during which examinations of the feces were made to determine if the animal's intestines were already infected with tetanus spores; and an experimental period, during which the animal received one feeding of tetanus spores. After this frequent examinations of the feces were made to determine for how long a time the spores continued to be voided.

Two full-grown guinea-pigs were put into metabolism cages provided with bottoms of coarse wire through which the feces dropped as quickly as passed. It was necessary to do this, as the pigs were found to be coprophagous because of their restricted diet. The feces were collected while fresh and planted in tissue broth tubes. Inoculations into young white rats were made after incubating the cultures for from one week to ten days.

The fore-period of the experiment lasted for eleven days. Three examinations of the feces were made during this time. All failed to show tetanus bacilli on animal inoculation. At the end of the fore-period, Guinea-pig 1 was given one feeding of 5 c.c. of a ten-day broth culture of tetanus spores. Guinea-pig 2 was used as a control to indicate whether or not after this single feeding of tetanus spores to Guinea-pig 1 there was any subsequent ingestion of tetanus spores with the food. The diet was restricted, consisting of bread and lettuce carefully washed in sterile water. Table 2 following shows the results for the experimental period.

TABLE 2

RESULTS OF EXPERIMENTAL PERIOD ON GUINEA-PIGS 1 AND 2

Guinea-Pig 1 (Experimental Animal)				Guinea-Pig 2 (Control Animal)	
Date	Weight, in Mg., of Feces Examined	Rat Inoculation	Mouse Inoculated with Culture Recovered from Rat Dying of Tetanus	Weight, in Mg., of Feces Examined	Rat Inoculation
March 12	300	0	..	300	0
March 13	400	0	..	300	0
March 15	600	0	..	400	0
March 18	1,000	+	+	1,000	0
March 20	1,500	0	..	500	0
March 24 *					

* Pig died but not of tetanus

The sign + indicates that inoculated animal died of tetanus; 0 indicates absence of tetanus reaction.

Just before Guinea-pig 1 was fed the tetanus culture, a capsule of powdered carmin was administered. The carmin was completely eliminated in the feces in twenty-four hours, but no tetanus spores could be demonstrated until the seventh day after feeding. That their appearance in sufficient numbers to indicate their presence in the feces at that time was due to some second accidental ingestion of spores, possibly with the food, is improbable since the control pig, kept under the same conditions and fed with the same food, should also have shown them in his feces had this happened. This was not the case, and it is more reasonable to suppose that the spores were being continually eliminated during these seven days, but in numbers too few to respond to cultivation.

On March 18, the day on which the tetanus spores appeared in the feces of Guinea-pig 1, a quantitative examination for the spores was made. One gram of feces was thoroughly shaken in a machine in 10 c.c. of broth. Dilutions containing the amounts of fecal material shown in Table 3 were made from this and after cultivation for one week were tested on white rats in the usual way.

TABLE 3

SHOWING RESULTS OF QUANTITATIVE EXAMINATION FOR TETANUS SPORES IN FECES OF GUINEA-PIG 1

Amount, in Mg., of Feces Tested	Result of Rat Inoculation	Result of Inoculation of Mouse with Cultures Recovered from Rat Dying of Tetanus
1,000	+	+
100	+	+
10	+	—
1	0	

The sign + indicates that the inoculated animal died of tetanus; — the failure to recover the tetanus bacillus from the rat dying of tetanus; 0 indicates absence of tetanus reaction.

In order to secure additional data, this entire feeding experiment was repeated on two normal guinea-pigs. As in the first case, the experiment was preceded by a fore-period of eleven days, during which the feces were collected and tested four times for tetanus. These four tests were all negative. The methods of examination and all other details were the same as before, and so only the tabulated results are given (Table 4).

In this last feeding experiment it will be noted that 10 c.c. of a spore-containing culture were given, whereas in the first case 5 c.c. were fed. The results are radically different, for in the second case the spores are eliminated continuously from the second day after feeding to the sixteenth, after which they disappear, while in the first case they appear once, on the seventh day after feeding. A possible explanation of this is that in the first case spores may have been

TABLE 4

Results of Experimental Period of Twenty-Five Days

Guinea-Pig 3 (Experimental Animal)			Guinea-Pig 4 (Control Animal)	
Fed 10 c.c. of a Twelve-Day Broth Culture of Tetanus Spores				
Date	Weight, in Mgs., of Feces Examined	Result of Rat Inoculation	Weight, in Mgs., of Feces Examined	Result of Rat Inoculation
April 8	500	+	600	0
April 10	500	+	400	0
April 11	550	+	300	0
April 12	300	+	450	0
April 23	500	+	400	0
April 30	400	0	250	0
May 3	500	0	400	0

The sign + indicates that the inoculated animal died of tetanus; — the failure to reaction. The feces of the control pig contained tetanus spores at no time during the experiment.

given off continuously in the feces in numbers too few to be detected by our methods, but if multiplication took place in the intestine it is conceivable that by the seventh day after feeding, sufficient numbers had begun to appear in the feces to respond to culture methods, as we have already suggested. In the second case, however, the initial dose of spores was sufficiently large to enable them to pass through in the feces from the very beginning. Unfortunately, Guinea-pig 1 died before further tests could be made. It is interesting to compare these results with those obtained by Sormani,[23] who found that tetanus spores persisted in the feces of a dog for sixteen days after feeding.

Two horses, "Nora" and "Lady," the feces of which had been shown to contain tetanus spores, were examined for the organism at different intervals for a period of six months. From the same stable two other horses, "Beauty" and "Floradora," which previously had not shown tetanus spores in their feces, were used as controls to show whether or not all the horses were subjected to fresh infections of their alimentary canals with tetanus spores during the period of observation. One gram of feces was tested 'and cultures were made on Smith's tissue broth and incubated for from six to ten days. The results of the experiment are given in Table 5.

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23. Centralbl. f. Bakteriol., 1891, 9, p. 421.

TABLE 5

RESULTS OF EXAMINATION OF TWO HORSES FROM WHICH TETANUS SPORES HAD BEEN OBTAINED BEFORE AND OF TWO HORES WHICH IN PREVIOUS TESTS GAVE NEGATIVE RESULTS

Date	Experimental Horses		Control Horses	
	"Nora"	"Lady"	"Beauty"	"Floradora"
January 20	+	+	0	0
January 27	+	+	0	0
February 3	+	+	0	0
February 10	+	0	0	0
February 20	+	Not tested	0	0
March 3	+	0	0	0
April 30	+	0	0	0
June 13	0	0	0	0
June 23	0	0	0	0
June 26	0	0	0	0
June 28	0	0	0	0
July 1	0	0	0	0
July 24	0	0	0	0

The sign + indicates that the rat inoculated died of tetanus. Dilutions showed that the feces of "Nora" contained tetanus spores in 0.1 gm., but not higher dilutions.

The results for "Nora" are extremely interesting, for they show that individual animals may harbor tetanus bacilli in their digestive tracts for many months following an initial infection. "Lady," on the other hand, appears to have eliminated the organism completely within fourteen days after the beginning of observation.

DISCUSSION OF RESULTS

If we refer to the results of the experiments in which tetanus spores were fed to guinea-pigs, it will be seen that one of the pigs so fed harbored spores for a period of sixteen days, after which the spores disappeared from the feces. Another pig showed spores on the seventh day after feeding, but unfortunately the experiment ended at that time because of the death of the animal. Tests on the two horses "Nora" and "Lady" show that the spores disappeared from the feces of "Lady" fourteen days after the beginning of the observations, while "Nora" harbored them continuously for four months, altho, here too, the organism eventually disappeared. The horses used as controls during this period of four months showed that no new infection at least of sufficient magnitude to be detected, took place. Quantitative tests have shown that tetanus spores may be found in the feces of animals carrying them in such amounts of feces as 0.1-0.01 gm., but not in smaller quantities. It is possible that our present-day methods of cultivation are responsible for failure to detect the spores in smaller amounts of feces, for to insure growth under artificial conditions it is generally necessary to transplant relatively large amounts of the tetanus cultures. It must be remembered also that the tetanus bacillus grows slowly, even under the most favorable conditions. Considering these facts, it seems inconceivable that tetanus spores can remain in the intestine even for sixteen days, being constantly voided in the feces during that time, unless they maintain a foothold there by multiplication. Surely this conclusion is strengthened by the observations on "Nora" which has been shown to harbor the organism, not for sixteen days only, but for four months. Why this one animal should carry the organism for such a long period, while the others were free from it, or had rid themselves of it so soon, is not clear, but the explanation is probably to be found in some difference in the nature of the intestinal contents, or the intestinal flora. The phenomenon of the human typhoid or cholera carrier is well known. It seems that we are dealing with a "tetanus carrier" in the case of "Nora" in so far as the distribution of tetanus spores is concerned. What the percentage of such "tetanus carriers" is to normal animals is a problem which remains to be investigated; but granting the existence of "tetanus carriers" the universal distribution of tetanus spores is at once explained.

SUMMARY

The tetanus bacillus appears in the intestines of many normal animals, especially of the herbivora, but apparently it seems impossible, with the methods at our disposal, to detect it there unless it is present in relatively large numbers.

Experimental evidence shows that the tetanus bacillus may multiply in the intestines of such animals.

The intestines, or rather the intestinal contents of certain individual animals, seem to offer especially favorable conditions for the growth of the tetanus bacillus; such animals are "tetanus carriers" comparable, in regard to the distribution of the organism, with typhoid or cholera carriers among human beings.

The presence of tetanus spores in soils, street dust, fresh vegetables, and on clothing and the skin is undoubtedly due to fecal contamination.